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Method Development and Validation of Simultaneous Estimation of Dolutegravir and Lamivudine in Synthetic Mixtures by UV-Visible Spectroscopy

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ABSTRACT

A simple, rapid, accurate and economical method has been developed for the simultaneous estimation of Dolutegravir and Lamivudine in synthetic mixture by using the Q –absorbance ratio method. Absorbance ratio method for the ratio of absorbance at two selected wavelengths, one which is an iso -absorptive point and other being λ_{max} of one of the two components. Dolutegravir and Lamivudine showed an absorptive point at 290 nm. The second wavelength used was 271 nm which is λ_{max} of Lamivudine. The linearity of the method was found to be in the range of 1-5 µg /ml of Dolutegravir and 6- 30 µg /ml of Lamivudine. The concentration of the drugs was determined by using a ratio of absorbance at iso -absorptive point and the λ_{max} of Lamivudine.

INTRODUCTION

Dolutegravir is an HIV- 1 antiviral agent. It inhibits *HIV integrase* by binding to the active site and blocking the strand transfer step of retroviral DNA integration in the host cell. The strand transfer step is essential in the inhibition of viral activity. Dolutegravir has a mean EC_{50} value of 0.5 nM (0.21ng/mL) to 2.1 nM (0.85ng/mL) in peripheral blood mononuclear cells (PBMCS) and MT- cells.

The side effects are Headache, nausea, upset stomach, diarrhea, trouble sleeping, cough, runny nose, skin rashes, unexplained weight loss, persistent muscle aches or weakness, joint pain, numbness or tingling of the hands/feet/arms/legs, severe tiredness, vision changes, abnormal liver function allergic reaction. Lamivudine is a synthetic nucleoside analog and is phosphorylated intracellularly to its active 5 – triphosphate metabolite, Lamivudine triphosphate (L-TP). This nucleoside analog is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. The lack of a 3 –OH group in the incorporated nucleoside analog prevents the formation of the 5 to 3 phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated.

The side effects are Abdominal or stomach discomfort, bloody urine, dark urine, decreased appetite, decreased frequency or amount of urine, diarrhea, a general feeling of discomfort, increased blood pressure, increased thirst, light-colored stools, loss of appetite, lower back or side pain, muscle pain or cramping, nausea and vomiting, sleepiness.

(A) (B)

Figure No. 1: Chemical structure of (A) Dolutegravir (B) Lamivudine

MATERIALS AND METHODS

Instruments

A UV-Visible double beam spectrophotometer Shimadzu 1800 Japan with 10mm quartz cells is used.

Shimadzu balance model AY 220 is used for weighing.

Materials

Pure drug sample of Dolutegravir and Lamivudine was kindly supplied as a gift sample by Mylan Laboratories Ltd., Hyderabad.

Chemicals and Reagents:

Distilled water, Methanol

Preparation of synthetic mixtures

Dolutegravir- 50

Lamivudine-300mg

MCC (Rolex chemical Industries, Mumbai)-10mg Magnesium Sulphate -10 mg

(Thermo fisher scientific, Mumbai)

Mannitol -10 mg

Sodium starch glycolate-15 mg

Sodium starch glycolate-10mg

Methods

Selection of wavelength

A 10mg of standard Dolutegravir and Lamivudine were weighed and transferred to 100ml separate volumetric flask, made up to volume with water contains 100 µg/ml of Dolutegravir and Lamivudine. The solution was scanned in the range of 200-400nm and

the maximum absorbance was noted at 258 nm for DOLU (Fig.1) and 271 nm for LAMI (Fig.2) against water as blank and the iso- the absorptive point was noted at 290nm. The overlay of the UV spectrum of both DOLU and LAMI were shown in (Fig.3).

Preparation of standard stock solution

An accurately weighed synthetic mixture equivalent to 5mg of Dolutegravir and 30mg of Lamivudine was transferred into a 50 ml standard flask. Dissolve the content in a little amount of methanol and was sonicated using ultra-sonicator for 5 minutes and made up to volume with water. Appropriate aliquots within the Beer's Law limit (100µg/ml DOLU and 600µg/ml LAMI) were analyzed by the proposed method.

Analysis of Synthetic mixtures

An accurately weighed synthetic mixture equivalent to 5mg of Dolutegravir and 30mg of Lamivudine was transferred into a 50 ml standard flask. Dissolve the content in a little amount of methanol and was sonicated using an ultra sonicator for 5 minutes and made up to volume with water then filtered using Whatman filter paper. Appropriate aliquots within the Beer's Law limit (100µg/ml DOLU and 600µg/ml LAMI) were analyzed by the proposed method. An aliquot of the sample solution, 0.3ml containing 3µg/ml of DOLU and 18µg/ml of LAM was pipetted into separate 10ml standard flask and the volume was made up with distilled water. The absorbance of the sample solution at 271 nm and 290 nm against water was measured. Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an iso - absorptive point and other being one of two components. The concentration of DOLU and LAM present in the mixture was calculated by using the following equation

$$C_X = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{a_{x1}}$$

$$C_Y = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A_1}{a_{y1}}$$

$$Q_M = A_2/A_1,\ Q_X = ax_2/ax1,\ Q_Y = ay_2/ay_1$$

Where C_X and C_Y are the concentration of Lamivudine and Dolutegravir, A1 and A2 are

the absorbances of the mixture at 271 nm and 290nm, ax_1 and ay_1 are absorptivities of Lamivudine and Dolutegravir at 271 and ax_2 and ay_2 at 290 nm respectively.

METHOD VALIDATION

The method was validated using ICH guidelines by determining the following parameters: Linearity, Accuracy, Precision, Robustness, Ruggedness, Precision, Detection limit and Quantification limit.

Linearity

From standard solutions, 0.1 ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml of aliquots are pipetted into 10ml volumetric flask and made up to mark with water to give concentrations of 1-5 µg/ml of Dolutegravir and 6-30 µg/ml of Lamivudine and linearity was measured by regression analysis.

Accuracy

The accuracy of the method was determined using a recovery analysis. A known quantity of the mixed pure drug was added to the accurately weighed synthetic mixture at 80%, 100%, and 120% levels. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation was calculated.

Precision

From the synthetic mixture, a particular concentration level 3 µg/ml of DOLU and 18 µg/ml Lamivudine were prepared and analyzed in three replicates during the same day (intra-day) and on three consecutive days (inter-day). And the percentage relative standard deviation was also calculated.

Robustness

The robustness of the method was estimated by introducing change in the solvent system from water to methanol.

Ruggedness

Ruggedness was determined by performing an analysis of the synthetic mixture following the recommended procedures by three different analysts.

Detection and quantification limit

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the intercept standard deviation and the curve slope.

$$LOD = 3.3\sigma/S \qquad LOQ = 10\sigma/S$$

RESULTS AND DISCUSSIONS

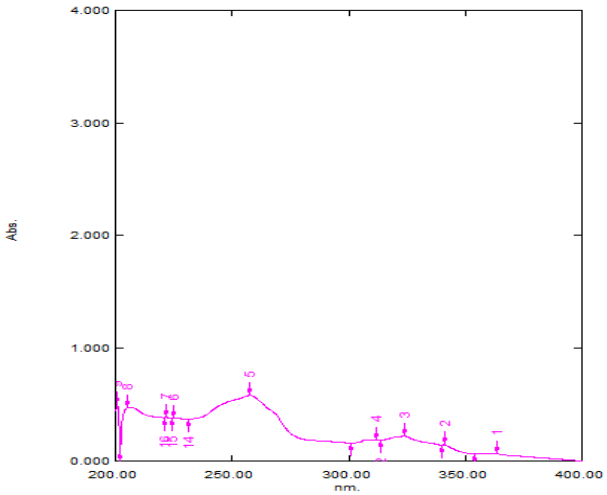

Figure No. 2: UV Spectrum of Dolutegravir

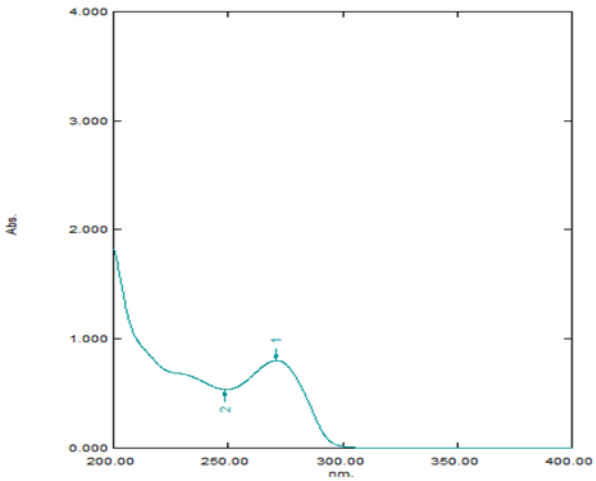

Figure No. 3: UV Spectrum of Lamivudine

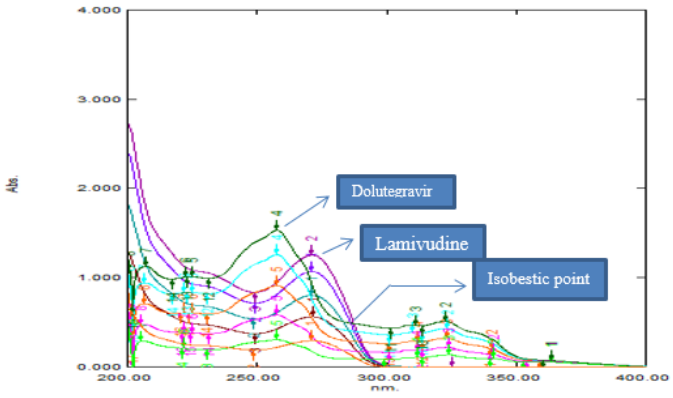

Figure No. 4: Overlay spectrum of Dolutegravir and Lamivudine

Table No. 1: Results of Assay of Synthetic mixtures

Drug	Label claim (mg)	Test conc (µg/ml)	Amount recovered (µg/ml)	% Assay	RSD
DOLU	50	3 3 3	3.0 3.0 3.1	100 100 100.3	1.7
LAM	300	18 18 18	18.1 18.2 18.1	100.5 101.1 100.5	1.8

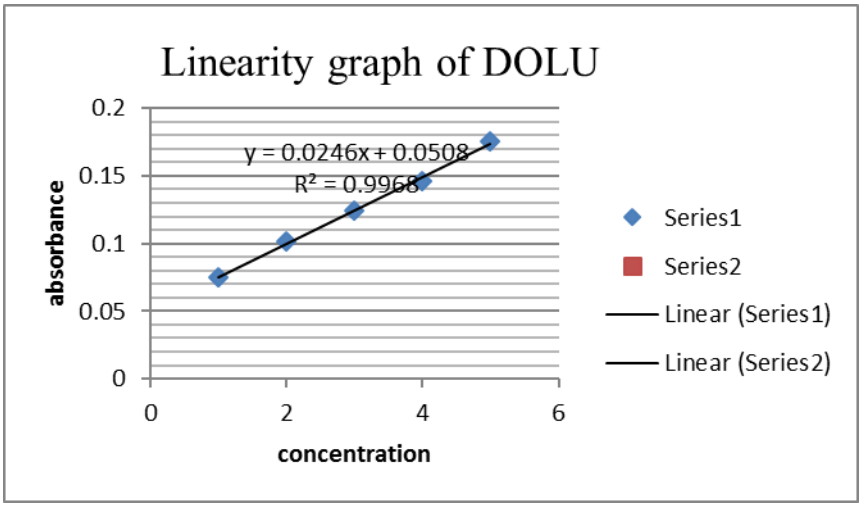

Figure No. 5: Linearity graph of Dolutegravir at 271 nm

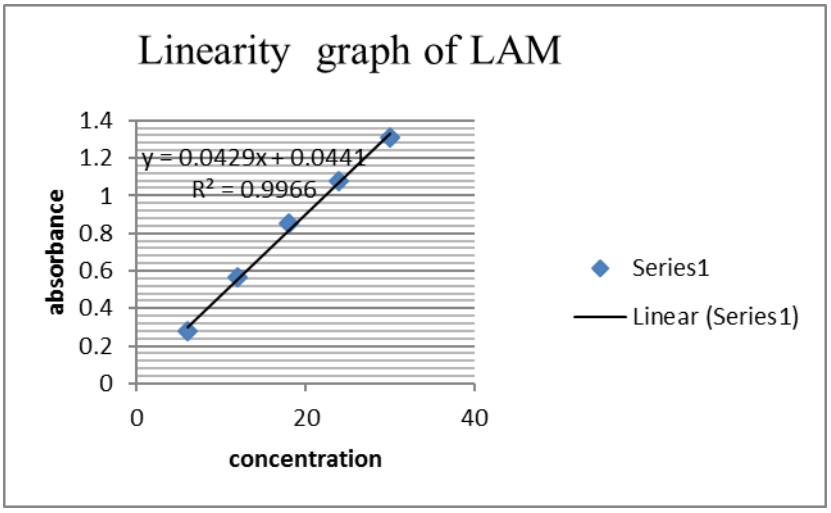

Figure No. 6: Linearity graph of Lamivudine at 271 nm

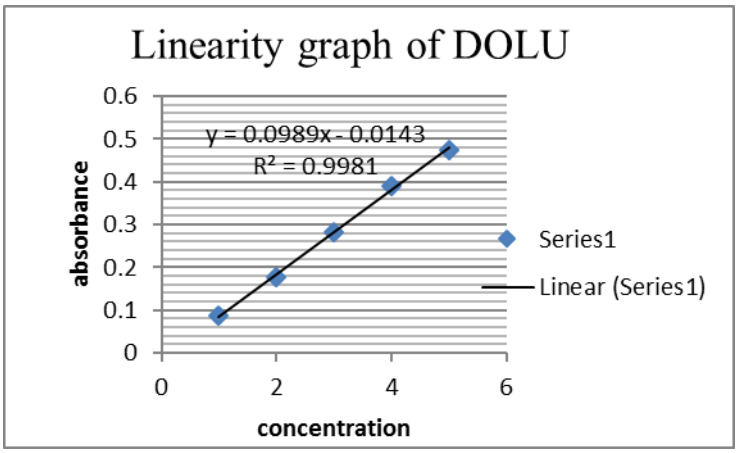

Figure No. 7: Linearity graph of Dolutegravir at 290 nm

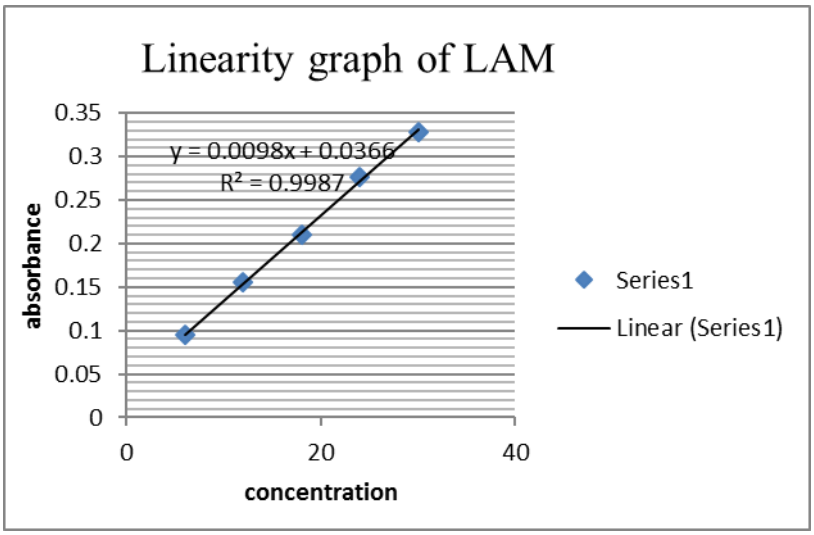

Figure No. 8: Linearity graph of Lamivudine at 290 nm

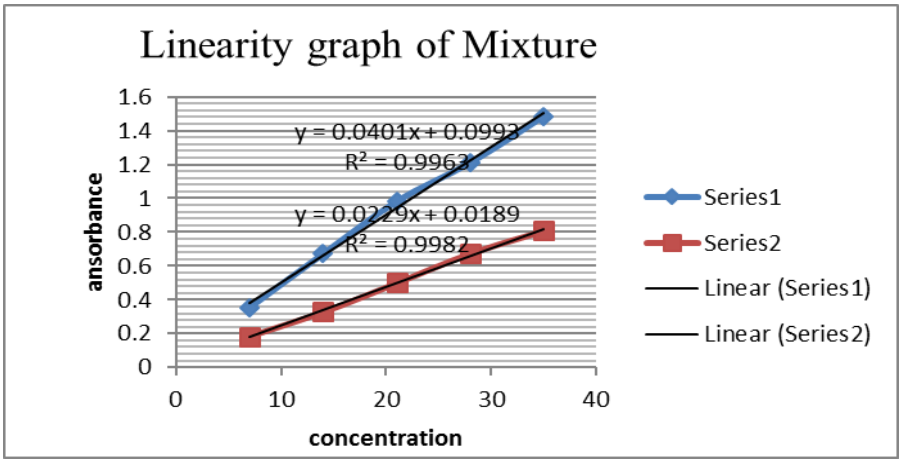

Figure No. 9: Linearity graph of the mixture at isosbestic point 290 nm

Table No. 2: Linearity result of Dolutegravir and Lamivudine at 271nm

DOLUTEGRAVIR		LAMIVUDINE	
Conc (µg/ml)	**Absorbance**	**Conc (µg/ml)**	**Absorbance**
1	0.075	6	0.278
2	0.102	12	0.565
3	0.124	18	0.850
4	0.146	24	1.076
5	0.176	30	1.308

Table No. 3: Linearity result of Dolutegravir and Lamivudine at 290 nm

DOLUTEGRAVIR		LAMIVUDINE	
Conc (µg/ml)	**Absorbance**	**Conc (µg/ml)**	**Absorbance**
1	0.088	6	0.095
2	0.176	12	0.155
3	0.282	18	0.210
4	0.391	24	0.277
5	0.475	30	0.328

Table No. 4: Linearity result mixture at 271 nm and 290 nm

MIXTURE AT 271 nm		MIXTURE AT 290 nm	
Conc (µg/ml)	**Absorbance**	**Conc (µg/ml)**	**Absorbance**
7	0.353	7	0.183
14	0.672	14	0.328
21	0.982	21	0.500
28	1.211	28	0.675
35	1.486	35	0.810

Table No. 5: Results of accuracy studies

Drug	Theoretical % target level	Amount added (µg)	Amount recovered (µg)	% Recovery	% RSD
DOLU	80	5.4	5.2	96.3 %	1.7
	100	6	6.1	101.66%	
	120	6.6	6.7	101.51%	
LAM	80	32.4	30.8	95.06%	1.8
	100	36	35.9	99.72%	
	120	39.6	40.10	101.26%	

Precision

Table no 6: Results of precision studies

Drug	Amount taken (µg/ml)	Intra day		Inter day	
		% content	% RSD	% content	% RSD
DOLU	3	96.4 96.8 96.6	1.6	96.3 96.5 96.7	1.6
LAM	18	98.9 98.8 98.6	1.4	98.6 98.5 98.8	1.4

Robustness

Table No. 7: Robustness results

Drug	Amount taken (µg/ml)	Amount recovered (µg)	% Content	% RSD
DOLU	3	3.1	103	1.8
		3.2	106	
		3.2	106	
LAM	18	18.6	103.3	1.7
		18.3	101.6	
		18.5	102.7	

Ruggedness

Table No. 8: Ruggedness results

Drug	Analyst	Amount taken (µg/ml)	Amount recovered (µg)	% Content	% RSD
DOLU	Analyst I	3	3.1	103	1.7
	Analyst II		3.1	103	
	Analyst III		3.2	106	
LAM	Analyst I	18	18.2	101	1.6
	Analyst II		18.3	102.2	
	Analyst III		18.3	101.6	

LOD and LOQ

Table No. 9: Results of LOD and LOQ

Dolutegravir		Lamivudine	
LOD (µg/ml)	**LOQ (µg/ml)**	**LOD (µg/ml)**	**LOQ (µg/ml)**
0.350 (271nm) 0.263(290 nm)	1.03 (271nm) 0.79(290 nm)	2.07(271nm) 1.27 (290 nm)	6.29(271nm) 3.8(290 nm)

CONCLUSION

The UV spectrophotometric Q- absorption ratio method was developed and validated for the simultaneous estimation of Dolutegravir and Lamivudine. The prescribed method was found to be simple, precise, accurate, robust, rugged and economic for the determination of Dolutegravir and Lamivudine in the synthetic mixture and its analytical method for combination is not available at any other, so this method is considered novel for combined form. The excipients and other additives present in the synthetic mixture do not interfere in the analysis of drug hence it can be convenient for routine quality control of drugs in the combine dosage form.

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